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Blood and Feather Concentrations of Toxic Elements in a Baltic and an Arctic Seabird Population

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ABSTRACT

We report blood and feather concentrations of elements in the Baltic Sea and Arctic population of common eiders (*Somateria mollissima*). The endangered Baltic Sea population of eiders was demonstrably affected by element pollution in the 1990s. While blood concentrations of Hg were higher in Baltic breeding eiders, blood Se, As and Cd concentrations were higher in Arctic eiders. Blood concentrations of Pb, Cr, Zn and Cu did not differ between the two populations. While blood Pb concentrations had declined in Baltic eiders since the 1990s, Hg concentrations had not declined, and were above concentrations associated with adverse oxidative effects in other bird species. Inconsistent with blood concentrations, feather concentrations suggested that Pb, Zn, and Cd exposure was higher in Baltic eiders, and that Hg exposure was higher in Arctic eiders. Our study thus emphasizes the need for comprehensive evaluation of toxic element status, covering the annual cycle of a species.

Key words: Common eiders, heavy metals, pollution, environmental exposure

Toxic elements are considered a major pollution problem because of their negative effects on humans and wildlife, including damage to liver and bones, birth defects, cancer, alteration of genetic and enzyme systems, and damage to nervous and immune systems (Koivula and Eeva, 2010; Nordberg et al., 2014). Although these elements occur naturally in the environment, human activities such as metal smelting, combustion, ore processing, battery manufacturing and recycling cause significant additional anthropogenic releases of elements. All organisms have to cope with element stress, either from exposure to non-essential toxic elements or from depletion, or excess of essential elements. The metals mercury (Hg), cadmium (Cd) and lead (Pb), as well as the metalloid arsenic (As) are toxic and non-essential, while the metals chromium (Cr), copper (Cu), and zinc (Zn), as well as the non-metal selenium (Se) are essential but become toxic at levels above background (Nordberg et al., 2014). Sediments are the ultimate sink for aquatically emitted elements, and coastal environments may have elevated levels of contaminants due to run off, point source pollution, river influxes, atmospheric transport and deposition (Nordberg et al., 2014). Sea ducks are large-bodied marine birds which primarily feed on benthic invertebrates. These species, notably the common eider (*Somateria mollissima*, hereafter eider), are therefore recognized as important indicators of ecological health and inshore marine pollution (Savinov et al., 2003; Goodale et al., 2008; Meattey et al., 2014). Their exposure to some elements may, therefore, be relatively high compared to other marine birds that are pelagic feeders (Henny et al., 1995). Furthermore, eiders are long-lived birds that may accumulate certain elements, and be chronically exposed to elevated levels of these elements (Wayland et al., 2001). The eider is therefore a good study species for contaminant monitoring in coastal

and inshore marine habitats (Franson et al., 2004; Mallory et al., 2004; Mallory et al., 2014; Provencher et al., 2014).

In the majority of avian studies, toxic element concentrations have been determined in tissue such as liver or kidney (Garcá-Fernández et al., 1996; Eisler, 2010; Shore et al., 2011; Binkowski and Meissner, 2013), a method that requires that the birds are killed. However, many marine bird species are considered threatened (Croxall et al., 2012), and non-destructive techniques using feather, blood and addled eggs are therefore preferable due to animal welfare concerns (Eisler, 2010). Furthermore, non-destructive sampling techniques make it possible to study survival rates in relation to contaminant exposure. Consequently, non-lethal sampling methods are increasingly being used to report toxic element concentrations (Wayland et al., 2007; Burger and Gochfeld, 2009). Blood element concentration is indicative of recent dietary exposure (Evers et al., 2008; Wayland and Scheuhammer, 2011), while feather element concentration indicates blood and body concentrations at the time of moulting (Markowski et al., 2013). The aim of the present study was to document blood and feather concentrations of toxic elements in the Baltic Sea and Svalbard eider population. The Baltic Sea has been considered one of the most polluted seas in the world (Fitzmaurice, 1993). It is a semi-enclosed, relatively shallow marine basin that captures and retains pollutants from numerous sources (HELCOM, 2010). During the last part of the 20th century the Baltic Sea has been exposed to large anthropogenic pollutant loads (Vallius, 2015b). The Baltic breeding eider population has declined by over 30% since the 1990s (Skov et al., 2011; Ekroos et al., 2012), and is considered endangered (Bird Life International, 2015). Main threats to this population include increased predation, human impact on marine habitats, oil pollution and changes in the quantity and/or quality of food

resources (Ekroos et al., 2012; HELCOM, 2013). Environmental analyses performed in the 1990s revealed elevated element concentrations throughout the Baltic Sea (Borg and Jonsson, 1996; Leivuori, 1998; Vallius, 1999). During the same time period Hollmèn et al. (1998) reported toxic levels of Se and Pb in blood and liver tissue of Baltic eiders, and dead eiders were diagnosed with Pb poisoning. Furthermore, Franson et al. (2000) reported a negative correlation between blood Pb concentration and delta-aminolevulinic acid dehydratase (ALAD, linked to development of anemia) in Baltic eiders. These studies suggest that toxic element exposure has posed a real threat to Baltic eiders in the past. More recent sediment analyses in the Baltic Sea indicate that toxic element levels have declined significantly during the last two decades (Vallius, 2014). However, there are indications that levels of certain toxic elements, such as Cd, Cu and Hg are still of concern in some areas of the Baltic Sea (Vallius, 2014; Vallius, 2015b). Thus, an updated documentation of toxic element concentrations in the Baltic Sea population of eiders is needed to assess the current toxic element exposure of this seabird population.

Compared to the Baltic Sea, Svalbard is considered a relatively clean area (Fenstad et al., 2016). However, sediment analyses indicate that there may be some Cd, Pb and Hg pollution in regions of the Norwegian Arctic (Lu et al., 2013). The major input of toxic elements into the Arctic, however, results from transport from more industrialized areas (AMAP, 2005), and evidence suggests that Hg deposition in the Arctic may increase due to increased global emissions (Riget et al., 2011). In contrast to the Baltic Sea population, the Svalbard eider population has remained stable over the last three decades (Hanssen et al., 2013). Toxic element levels in Svalbard eiders have, to our knowledge, only been reported in tissues such as liver,

kidney or muscle (Norheim and Kjos-Hanssen, 1984; Norheim and Borch-Iohnsen, 1990; Savinov et al., 2003). The reported liver concentrations of Cd, Cu and Se in Svalbard eiders in the 1980s and 1990s were comparable with Baltic eiders, while liver Hg and possibly As concentrations appeared to be higher in Baltic eiders. Liver Pb concentrations have not been reported in Svalbard eiders (Norheim, 1987; Norheim and Borch-Iohnsen, 1990; Hollmèn et al., 1998; Savinov et al., 2003). Sediment analysis from the Baltic Sea (Gulf of Finland) and Svalbard (Kongsfjorden) indicates that the concentrations of Cd and Hg are higher in the Baltic Sea compared to Svalbard, but for other toxic elements, sediment concentrations may be comparable between the two locations (Grotti et al., 2013; Lu et al., 2013; Vallius, 2009, 2015a).

The objectives of the present study were to determine and compare blood and feather concentrations of 8 elements in Baltic and Svalbard eiders. The blood element concentrations in Baltic eiders were also compared and evaluated in relation to previously reported blood concentrations in this population. Furthermore, the toxicological relevance of the blood element concentrations in the two populations was evaluated, based on previously reported adverse effects and threshold levels in birds.

Blood and underlying body feather samples were obtained from incubating female eiders in Tvärminne (N=28), Finland (~59°84'N, 23°21'E) and at Storholmen, Kongsfjorden (N=29), Svalbard (78°56'N, 12°13'E), in 2011. Baltic eiders migrate to Denmark, Germany and the Netherlands during winter (Lehikoinen et al., 2008),

whereas Svalbard eiders migrate to Iceland and northern Norway (Figure 1) (Hanssen et al., 2016). Body feathers are likely moulted in these areas.

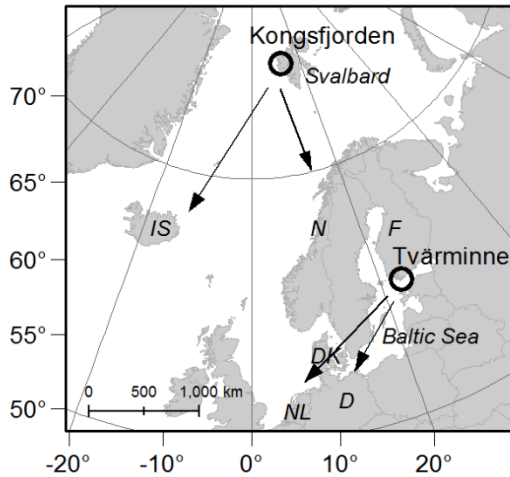


Figure 1: Breeding locations (circles) of the Svalbard (Kongsfjorden, 78°56'N, 12°13'E) and the Baltic (Tvärminne, Finland (F), 59°84'N, 23°21'E) eider populations. Arrows show the wintering areas in Iceland (IS) and northern Norway (N) for the Svalbard eiders (Hanssen et al., 2016), and Denmark (DK), the Netherlands (NL) and Germany (D) for the Baltic eiders (Lehikoinen et al., 2008). The map is in a North Pole stereographic projection.

The females were caught on the nest using a fishing rod with a nylon snare at the end, or with hand-nets. Female eiders fast during their incubation period and incubation stage may affect blood levels of certain elements (Franson et al., 2000; Wayland and Scheuhammer, 2011). Thus, females were only sampled if the clutch had hatched or was near hatching based on egg floatation (Kilpi and Lindström, 1997). Body mass (to the nearest 10 g) was recorded using a spring balance (Pesola Medio-Line 42500, Ecotone-Poland, 2500 g). Blood (8-10 mL) was sampled from the

jugular vein using a heparinised syringe. The blood was transported to the field station within six hours, and two mL whole blood was frozen (-20°C) for subsequent element analyses. Underlying body feathers were sampled from the back of the females and packed in enclosed transparent plastic bags. Blood samples and feathers were transported to the laboratory at the Norwegian University of Science and Technology (NTNU), Trondheim, at the end of field season. The study complies with the Norwegian and Finnish regulation on animal experimentation, and permission for field work was granted by the Governor of Svalbard and the local authorities in Finland (Animal Experiment Board/State Provincial Office of Southern Finland, permit number ESLH-2009-02969/Ym-23).

Blood and feather samples were analysed for concentrations of the elements Hg, Se, Pb, Cd, As, Cr, Zn and Cu using High Resolution Inductively Coupled Plasma Mass Spectrometry (HR-ICP-MS, Thermo Electronic Corporation, Waltham, MA, USA) at the Department of Chemistry, NTNU.

For the detection of the elements, approximately 500 mg blood was transferred to acid washed Teflon tubes, designed for UltraClave, and added to 0.5 mL 50% Scanpure nitric acid (HNO₃ ultra-pure grade, 14.4 M) for digestion. For the feather samples, approximately 30 mg of feathers was added two mL 50% HNO₃ for digestion. The samples were digested using a high pressure microwave system, UltraClave (Milestone, Shelton, CT, USA) over two hours with temperature up to 240 °C and pressure of 160 bar. Blood samples were diluted to 12 mL and feather samples were diluted to 24 mL with ion exchanged Milli-Q-water before element analysis.

To assure the quality of the analysis, four reference material samples (seronorm, Trace Elements Whole Blood L-1, LOT MR4206, REF 201505, Sero, Billingstad, Norway) were analysed with the blood samples. Also, four reference samples (tea leaves, GBW07601- GBW07605, Institute of Geophysical and Geochemical Exploration, Langfang, China) were analysed with the feather samples. Two replicates of four of the blood samples were analysed in different runs, and three blank samples accompanied every run of the analysis. The analysed reference material was within the approved range values for all analysed elements. The results were corrected from blank samples and the detection limits ranged between 1 - 200 µg/kg for feathers and 0.02 – 3.6 µg/kg for blood for the analysed elements. Concentrations were above the detection limit, with the exception of four individuals with feather Cr concentrations and four individuals with blood Cr concentrations below the detection limit (6 and 0.1 µg/kg, respectively). Negative Cr concentrations were set to zero, and positive Cr concentrations below the detection limit were set to 50% of the detection limit. Element concentrations are presented as µg/kg wet weight (ww).

Statistical analyses were carried out using the programme R, version 3.2.3 (R Development Core Team., 2016). Separate linear models (lm function), with blood concentration as dependent variable, and population (factor with two levels, Baltic Sea and Svalbard), feather element concentration and female body mass as independent variables were used to test differences in blood concentrations between the two populations. The two latter covariates were included to test whether female body mass and feather element concentrations affected the concentrations of elements in blood. Independent variables with p values > 0.1 were removed from the

starting model. Finally, separate linear models for each element, with feather element concentration as dependent variable and population as independent variable were used to test differences in feather concentrations between the two populations.

A Se rich diet can prevent toxic effects from Hg (Ralston et al., 2007; Sørmo et al., 2011) because Se binds and inactivates Hg (Dyrssen and Wedborg, 1991), and thereby counteracts its toxic effects (Sørmo et al., 2011; Mulder et al., 2012). The molar ratio between Se and Hg in blood was calculated for both eider populations.

Diagnostic plots in R (Residuals vs. Fitted, Normal QQ, Scale-Location and Residuals vs. Leverage plot) were used to assess whether the data sufficiently met the assumptions of a linear model (normal distribution of residuals, linearity, equal variance). All blood and feather concentrations, with the exception of feather concentration of Cu, were \log^e -transformed prior to statistical analyses to satisfy the assumptions of parametric tests. All tests were two-tailed and significance was set at $p < 0.05$.

Blood concentrations of Hg ($t_{55} = -4$, $p = 0.0001$) were significantly higher in Baltic eiders compared to Svalbard eiders (Figure 2), whereas blood concentrations of Se ($t_{54} = 6$, $p < 0.0001$), As ($t_{55} = 3$, $p = 0.005$) and Cd ($t_{54} = 7$, $p < 0.0001$) were higher in Svalbard eiders. The blood concentrations of Pb ($t_{55} = 1$, $p = 0.3$), Cr ($t_{54} = -0.5$, $p = 0.6$), Zn ($t_{54} = -1$, $p = 0.3$) and Cu ($t_{54} = 1$, $p = 0.3$) did not differ between the two eider populations (Figure 2). Blood element concentrations were not affected by female body mass ($p > 0.3$), with the exception of a positive relationship between body mass and the blood concentration of Cu and Zn ($p < 0.05$).

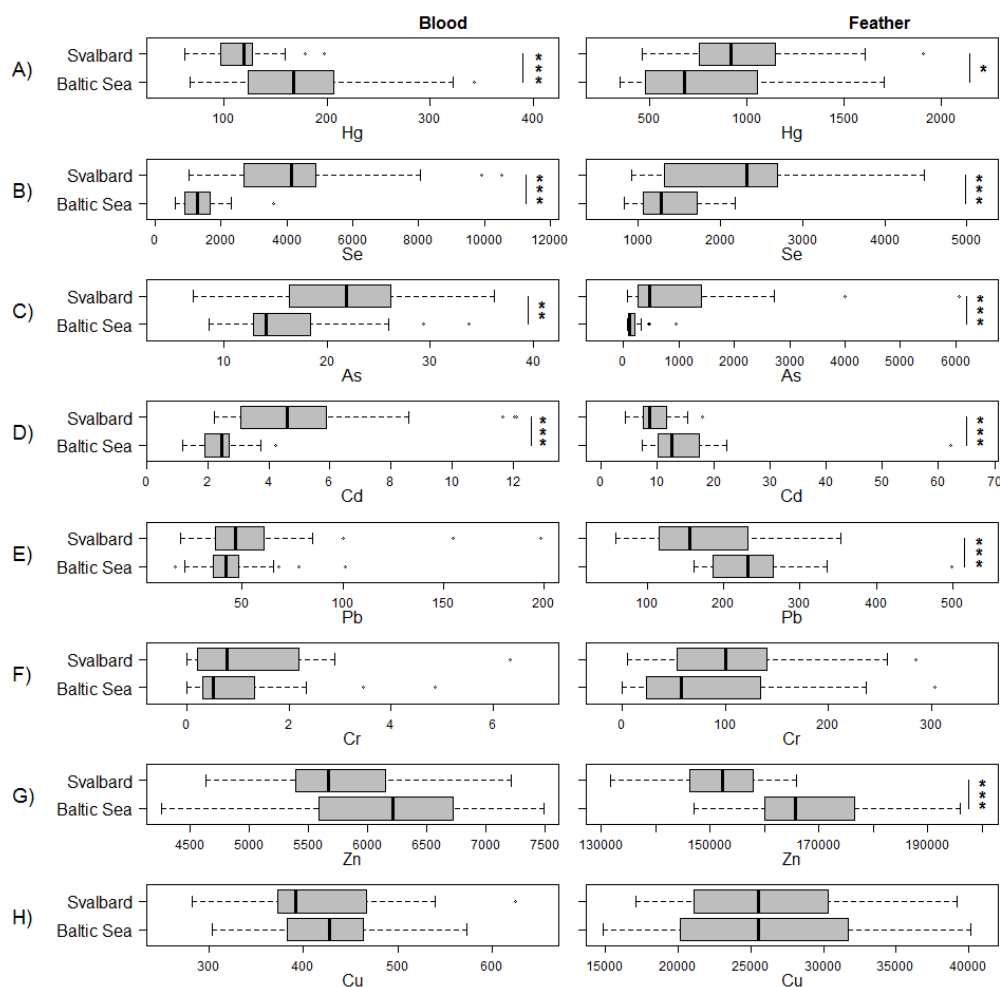


Figure 2: Box plots with blood (left) and feather (right) concentrations (µg/kg ww) of A) mercury (Hg), B) selenium (Se), C) arsenic (As), D) cadmium (Cd), E) lead (Pb), F) chromium (Cr), G) zinc (Zn) and H) copper (Cu) in female eiders from Svalbard (upper box) and the Baltic Sea (lower box). The plots show medians (thick vertical lines in boxes) along with the interquartile range (IQR, box), maximum and minimum values within 1.5 IQR (whiskers) and values outside IQR (outliers).

P-values from linear models (see text) are indicated by stars (*) to the right of the boxes, where $p < 0.001$ is indicated by ***, $p < 0.01$ by ** and $p < 0.5$ by *.

The mean blood concentration of Hg was 1.5 times higher in the Baltic eiders than in the Svalbard eiders. The highest individual blood concentration of Hg in the Baltic

population was 343 µg/kg. Furthermore, the Svalbard eiders had three times higher mean blood concentrations of Se compared to the Baltic eiders. Thus, there were large differences in the molar relationships of Se and Hg in the two populations. While the Se:Hg molar ratio was 92 (55:0.6 (µmol/kg)) in blood of Svalbard eiders, it was 19 (17:0.9 (µmol/kg)) in the blood of Baltic eiders.

Inconsistent with blood concentrations (Figure 2), the feather concentrations of Hg ($t_{55} = 2$, $p = 0.047$) were higher in Svalbard eiders than in Baltic eiders (Figure 2). Although blood concentrations of Cd were significantly higher in Svalbard ($t_{54} = 7$, $p < 0.0001$), the feather concentrations were twice as high in Baltic eiders ($t_{55} = -4$, $p < 0.0001$). Furthermore, the feather concentrations of Pb were 1.4 times higher in Baltic eiders ($t_{55} = -4$, $p = 0.0002$), and feather concentrations of Zn were 1.1 times higher in Baltic eiders compared to Svalbard eiders ($t_{55} = -5$, $p < 0.0001$) (Figure 2). Consistent with the patterns in blood, feather concentrations of Se ($t_{55} = 4$, $p < 0.0001$) and As ($t_{55} = 6$, $p < 0.0001$) were higher in Svalbard compared to Baltic eiders (Figure 2). As for blood, there were no significant differences in the feather concentrations of Cu and Cr between the two populations ($p > 0.1$, Figure 2).

For Se ($t_{54} = 4$, $p = 0.01$) and Cr ($t_{54} = 2$, $p = 0.02$), there was a significant positive correlation between the blood and feather concentration. However, for the remaining elements, blood and feather concentrations did not correlate with each other ($p > 0.05$).

This study reports blood element concentrations from the Arctic eider population in Svalbard for the first time, and provides up-dated information on element exposure in the Baltic population, which was presumably exposed to potentially toxic concentrations in the 1990s (Hollmèn et al., 1998; Franson et al., 2000). The

differences in blood and feather element concentrations between the two populations in the present study suggest different exposure patterns at the breeding and moulting locations of these two populations.

Blood concentrations of Hg, Se, As and Cd, indicating recent exposure, differed between the Baltic Sea and Svalbard eider populations. In accordance with reported sediment concentrations of elements at the two locations (Vallius 2009; Grotti et al., 2013; Lu et al., 2013; Vallius 2015a), blood concentrations of Hg were significantly higher in Baltic eiders compared to Svalbard eiders. This is consistent with previous reports indicating that Hg levels in Baltic biota, such as in fish and blue mussels (*Mytilus edulis*), are far above the assumed threshold levels for natural background concentrations, associated with non-polluted areas (HELCOM, 2010). The blood concentrations of Hg in the Baltic eiders were comparable to those reported in eiders from the same location in 1997 and 1998 (maximum concentration of 0.22 - 0.31 ppm (= 220 - 310 µg/kg ww) (Franson et al., 2000; Hollmèn et al., 1998), suggesting that the bioavailable environmental concentrations of Hg in the Baltic Sea have not declined during the last two decades. The blood Hg concentrations in Baltic eiders (67 – 343 µg/kg) were in the same range as in eiders in the Canadian Arctic in 1998 (140 – 370 µg/kg) (Wayland et al., 2001), whereas the blood concentrations in the Svalbard eiders were lower (62 – 197 µg/kg). Because there are few reports on blood concentrations of toxic elements in eiders, it is not possible to apply results from the present study to provide a more comprehensive overview of spatial variation in Hg levels in arctic eider populations. However, in a recent study, Lovvorn et al. (2013) summed up previously reported hepatic Hg concentrations in eiders from Svalbard, Western Greenland, East Canada and Finland. Although these data

originate from the 1980s, these results indicate that during this period, Hg concentrations were lowest in the East Canadian Arctic (Wayland et al., 2001) and Svalbard (Savinov et al., 2003), intermediate in eiders from Western Greenland (Dietz et al., 1996), and highest in eiders from Finland (Hollmen et al., 1998).

Contrary to Hg, blood concentrations of Cd, Se and As were greater in Svalbard compared to Baltic eiders. Reported sediment concentrations from relevant locations in the Baltic Sea and Svalbard might indicate that exposure levels to Cd are higher in the Baltic Sea (Vallius 2009; Grotti et al., 2013; Lu et al., 2013; Vallius 2015a). Furthermore, in most sites in the Baltic Sea, Cd concentrations in blue mussel are higher than the estimated natural background levels associated with no anthropogenic pollution (HELCOM, 2010). Nevertheless, blood Cd concentrations in both eider populations were far below the blood levels suggested to be caused by exposure to natural background Cd levels ($< 26 \mu\text{g/dl}$ ($\sim 260 \mu\text{g/kg}$) (Cutnell and Johnson, 2004)), and in accordance with the commonly reported blood concentrations of Cd ($< 5 \text{ ng/mL}$) in wild birds (Wayland and Scheuhammer, 2011).

Blood concentrations of Se in both eider populations were comparable to those reported in the Baltic eiders in the 1990s (1180 – 3390 $\mu\text{g/kg ww}$ and 300 – 9250 $\mu\text{g/kg ww}$) (Hollmèn et al., 1998; Franson et al., 2000). Although Se concentrations below 0.4 mg/L ww ($\sim 0.4 \text{ mg/kg}$ (400 $\mu\text{g/kg}$) (Cutnell and Johnson, 2004)) are considered to represent background levels in whole blood of non-marine birds, marine birds in non-polluted areas tend to have greater Se concentrations in their blood (Harry and Gary, 2011).

Blood concentrations of As were higher in Svalbard compared to Baltic eiders. However, As levels tend to be higher in marine organisms (Kunito et al., 2008), and the Baltic eiders may have lower concentrations because they feed in brackish

water. The majority of avian studies reporting As levels have focused on tissue levels (Eisler, 2010; Sanchez-Virosta et al., 2015).

Blood concentrations of Pb, Cr, Zn and Cu did not differ between the two eider populations. Blood Pb concentrations in the present study were comparable to the lowest blood concentrations measured in Baltic eiders in 1997 and 1998 (20 - 520 µg/kg ww) (Franson et al., 2000). Hence, the present study suggests that for Baltic eiders the exposure to Pb has decreased during the last two decades. Lead additives in petroleum has been the major source of atmospheric Pb and contributed significantly to Pb levels in biota (Tong et al., 2000). Lead additives in petroleum were phased out during the 1990s, and completely prohibited in the European Union by 2005 (EU, 1998). Furthermore, a total ban of lead shots in waterfowl hunting in Finland was introduced in 1996 (Avery and Watson, 2009). Thus, exposure to Pb in Baltic eiders has likely decreased due to both reduced emissions of Pb containing petroleum products and legislative restrictions on the use of lead ammunition during the last two decades, with a concomitant decrease in blood concentrations of Pb.

There are limited published data on blood concentrations of Cr and Zn in different waterbird species (Eisler, 2010; Binkowski and Meissner, 2013). The blood concentrations of Cr in Svalbard and Baltic eiders were lower than in mallards (*Anas platyrhynchos*) from urban areas in Poland (0-0.4 µg/g ww, (Binkowski and Meissner, 2013)), suggesting that Baltic and Svalbard eiders are likely not exposed to high levels of Cr. The blood concentrations of Zn in Baltic and Svalbard eiders were generally higher than the blood concentrations reported in 11 wild waterbird species in the area around Doñana National Park, Spain, following a toxic spill from the Aznalcóllar mine (0.3 – 8.6 mg/L (Benito et al., 1999)= ~ 300 – 8600 µg/kg (Cutnell

and Johnson, 2004)), but lower than in the blood of a Baltic top predator, the white tailed eagle in the 1980s (*Haliaeetus albicilla*, 7500 µg/kg (Falandysz et al., 1988)).

Reference plasma Cu concentrations of non-exposed birds have been suggested to range between 0.07 – 0.19 ppm (Osofsky et al., 2001). Red blood cell concentration of Cu in birds may be approximately 3 times that of plasma concentrations (Evans and Wiederanders, 1967). Hence, blood Cu concentrations of Baltic and Svalbard eiders were comparable to the suggested reference levels of non-exposed birds of approximately 200 – 600 µg/kg (Osofsky et al., 2001).

In eiders, flight feathers are replaced once a year, and the moulting of body feathers is divided into pre-nuptial body moult and post-nuptial body moult. The female pre-nuptial moult may extend until March, depending on latitude (Waltho and Coulson, 2015). The feather is connected to blood vessels during growth and may ideally be used to indicate circulating concentrations of elements at feather formation (Burger and Gochfeld, 1992; Markowski et al., 2013). However, external contamination of certain elements may occur (Appelquist et al., 1984; Goede and de Bruin, 1984; Weyers et al., 1988), at least on the exposed outermost feathers (Jaspers et al., 2004). Secretion products from the birds, such as preening oils, might also contribute to the element concentrations measured in feathers (Goede and de Bruin, 1984; Pilastro et al., 1993). Although the feathers were not washed before analysis, external atmospheric contamination has likely not contributed extensively in the present study, as underlying, hidden body feathers were collected, and we have no reason to expect any systematic bias due to potential external atmospheric contamination of feather samples.

Contrary to the pattern in blood, Hg concentrations were slightly, but significantly ($p = 0.047$) higher in feathers of Svalbard eiders, and Cd concentrations were twice as high in feathers of Baltic eiders (Figure 2). Furthermore, feather concentrations of Pb and Zn were significantly higher in Baltic eiders, although the blood concentration of Pb and Zn did not differ between the two populations. Thus, the differences in the element concentrations in blood and feathers within the two populations, suggest that exposure to Hg may be greater in Svalbard eiders during moulting, while body burden and exposure to Cd, Pb and Zn may be greater in Baltic eiders during moulting. Hence, the present study clearly indicates that these eiders breed and moult at different locations, which expose them to seasonally variable loads of toxic elements. It should also be noted that the Hg concentrations in the body feathers of the present eiders (Baltic: 0.3 – 1.7 $\mu\text{g/g}$, Svalbard: 0.5 – 1.9 $\mu\text{g/g}$) were somewhat higher than reported in primary feathers of eiders from the Canadian Arctic (0.24 – 0.95 $\mu\text{g/kg}$) (Mallory et al., 2015). Although this difference may be due to the different feather-types analysed, it may indicate that eiders from the Canadian Arctic winter in regions with less bioavailable Hg than the Svalbard and Baltic eiders.

Although adverse effect thresholds for Hg in blood of sea ducks have not been determined (Meatley et al., 2014), the concentrations in the present study (62 – 343 $\mu\text{g/kg ww}$) were lower than most previously reported threshold concentrations for toxic effects in birds (Wolfe et al., 1998; Scheuhammer et al., 2007). For instance, 700 $\mu\text{g/kg}$ and 3000 $\mu\text{g/kg ww}$ resulted in reproductive effects in common loons (*Gavia immer*) (Evers et al., 2008) and Carolina wrens (*Thryothorus ludovicianus*), respectively. However, in a recent study, Espin et al. (2014b) found large increases in lipid peroxidation at blood concentrations of 3 and 10 $\mu\text{g/dl ww}$ (~ 30 and ~ 100

µg/kg (Cutnell and Johnson, 2004)) in eagle owls (*Bubo bubo*). Furthermore, in griffon vultures (*Gyps fulvus*), blood Hg concentrations of 3 µg/dl ww (~ 30 µg/kg (Cutnell and Johnson, 2004)) were associated with a 10% increase in superoxide dismutase (SOD) activity (Espín et al., 2014a). Thus, in particular Baltic eiders, with greater blood Hg concentrations than Svalbard eiders, may risk adverse oxidative effects from Hg exposure.

The toxic interactions between Se and Hg are complex and not well understood (Shore et al., 2011). Evidence suggests that correlations between Se and Hg, and Se protection from Hg toxicity, at least in tissue, are only relevant for the most exposed seabird species where Se:Hg molar ratios approach 1 (Kim et al., 1996). The blood molar relationship between Se and Hg was 92 in Svalbard eiders compared to 19 in Baltic eiders. However, Hg sensitivity varies between avian species (Heinz et al., 2009), and the sensitivity of eiders to Hg compared to other seabird species is unknown (Meatley et al., 2014). Thus, potential effects from Hg exposure in eiders warrants further studies.

Although Se may protect against the adverse effects of Hg and is an essential element, high Se concentrations may be toxic (Harry and Gary, 2011). Both eider populations had blood Se concentrations considered adequate for nutritional needs in birds (0.13-0.20 mg/kg ww) (Harry and Gary, 2011), and were therefore not at risk of Se depletion. Although Hollmén et al. (1998) reported similar blood Se concentrations in eiders to be potentially toxic, blood Se concentrations of Baltic and Svalbard eiders were below most reported blood concentrations causing effects on adult survival, body mass or breeding probability in experimental studies on birds (Harry and Gary, 2011). Furthermore, no correlations were found between blood Se concentrations and survival in eiders from Alaska (Wilson et al., 2007), where blood

levels tended to be higher than in the present study (1500-15700 µg/kg ww vs. 600-10500 µg/kg ww). In that particular study it was suggested that eiders living in environments with high Se exposure may have higher Se tolerance. In addition, eiders also appear to be more tolerant to Se than other waterbirds, such as mallards (Harry and Gary, 2011). Hence, Svalbard and Baltic eiders are likely not exposed to toxic levels of Se.

A blood Pb concentration ≥ 200 µg/kg is considered above the background level, based on concentrations in non-exposed wild birds and on the concentrations at which clinical effects and mortality may occur (Sanderson and Bellrose, 1986; Franson and Deborah, 2011). A blood Pb concentration of 500 µg/kg is considered sub-lethal but toxic (Sanderson and Bellrose, 1986; Franson and Deborah, 2011). Inhibition of ALAD in birds has been reported at blood Pb concentrations of 50 µg/kg (Franson and Deborah, 2011). Five of the 28 Baltic eiders and 13 of the 29 Svalbard eiders had Pb concentrations above 50 µg/kg. It has been estimated that concentrations of 150-200 µg Pb/kg lead to a reduction in ALAD activity by 50% (Franson and Deborah, 2011). In the present study, two of the 29 Svalbard eiders had concentrations within this range. Although the current blood concentrations of Pb were below these toxic threshold levels in most individuals from both populations, almost 50% of the Svalbard eiders had concentrations that were higher than the lowest threshold level. This may indicate that Pb levels are close to threshold levels for ALAD inhibition in both populations. However, birds appear to have some tolerance for ALAD inhibition before a reduction in haemoglobin concentration occurs (Franson and Deborah, 2011). In the study of Franson et al. (2000), the maximum blood concentration of Baltic eiders was 520 µg/kg ww, indicating that eiders sampled at that time had blood concentrations of Pb above background, and that

some females were also in danger of Pb poisoning. Although Pb levels have decreased since the 1990s, and most of the Baltic and Svalbard eiders are currently not at risk of Pb poisoning, more research may be needed to conclude on the potential effects of Pb on ALAD inhibition in these eiders.

Cadmium accumulates in the liver and kidney of birds, and these are the tissues for which there is most information available on concentration-effect relationships (Wayland and Scheuhammer, 2011). Furthermore, Cd has no known toxicity threshold in avian blood (Wayland et al., 2008). In North American white-winged scoters (*Melanitta fusca*, 0.3-30.7 ng/mL (~0.3-30.7 µg/kg)) and king eiders (*Somateria spectabilis*, 2-76 ng/mL), no relationship was, however, found between blood levels of Cd and survival (Wayland et al., 2007; Wayland et al., 2008). Hence, Svalbard and Baltic eiders are exposed to low levels of Cd and are likely not at risk of toxic effects.

Evaluating the toxicity relevance of blood As, Zn and Cu concentrations is difficult because little data exist, and experimental studies with birds have not reported blood levels (Eisler, 1993; Osofsky et al., 2001; Eisler, 2010; Sanchez-Virosta et al., 2015). Zinc poisoning has, however, been documented in birds, but usually as a direct result of ingesting Zn containing products (Eisler, 1993). Plasma concentrations after poisoning with Zn were as high as 15.5 mg/L (~ 15500 µg/kg, (Eisler, 1993; Cutnell and Johnson, 2004), which is twice as high as the highest Zn concentration in whole blood in eiders in the present study.

In summary, Baltic and Svalbard eiders are exposed to element levels below the blood concentrations associated with toxic effects in birds. The present study also suggests that in general, element exposure has declined in the Baltic Sea since the 1990s. However, Hg exposure apparently remains similar in Baltic eiders, and the

blood concentrations of Hg exceed thresholds for adverse oxidative effects reported in other avian species. The contrasting patterns between blood and feather concentrations of particularly Hg and Cd, however, emphasize the need for year-round monitoring of exposure to elements to comprehensively assess toxic element status.

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